Nuclease

Week 7

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Extraction of pSB1K3-T7-Nuc from transformed BL21(DE3) with PCR analysis

Responsible

Ellinor Lindholm, Oscar He and Maren Maanja

Protocols used

Plasmid Extraction
PCR Amplification (Q5)
Gel electrophoresis

Modifications and comments to protocols

Plasmid Extraction - Step 1:	centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
Plasmid Extraction - Step 7:	not performed for half of the samples
Plasmid Extraction - Step 10:	DNA elution using ddH_2O
PCR Amplification (Q5):	1 μl DNA sample used in PCR mix.
PCR Amplification (Q5) primers:	VR (reverse)
	VF2 (forward)

Experimental Setup

Table 1: Volumes of each liquid cell over night culture used for DNA plasmid extraction.

Sample	Volumes [μl]
Nuc BL21(DE3) 1	3
Nuc BL21(DE3) 2	3
Nuc BL21(DE3) 3	3
Nuc BL21(DE3) 4	3
Nuc BL21(DE3) 1 (No PB)	3
Nuc BL21(DE3) 2 (No PB)	3
Nuc BL21(DE3) 3 (No PB)	3
Nuc BL21(DE3) 4 (No PB)	3

Table 2: Calculated concentration of sample plasmid in PCR mix.

Sample	Concentration [ng/μl]
Nuc BL21 1	3.9
Nuc BL21 2	4.3
Nuc BL21 3	3.3
Nuc BL21 4	3.2

Sample Calculation

The final concentration of plasmid sample in PCR mix

$$Final\ concentration = \frac{Initial\ concentration}{Dilution\ factor} \quad (1)$$

Results and Conclusions

Table 3: Concentrations of extracted plasmid DNA measured using NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc BL21(DE3) 1	96.3
Nuc BL21(DE3) 2	108.5
Nuc BL21(DE3) 3	81.7
Nuc BL21(DE3) 4	79.7
Nuc BL21(DE3) 1 (No PB)	124.7
Nuc BL21(DE3) 2 (No PB)	129.9
Nuc BL21(DE3) 3 (No PB)	93.5
Nuc BL21(DE3) 4 (No PB)	106.6

The DNA Extraction was successful as seen in Table 3. The concentrations were higher when not performing step 7 (referred to as No PB) during DNA extraction.

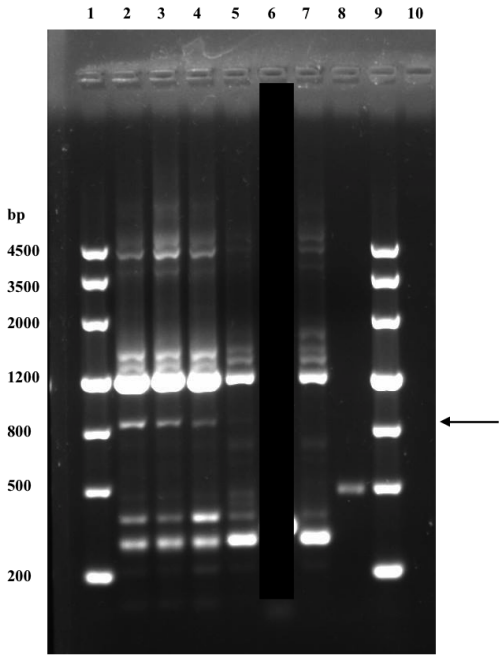

Figure 1: Result of the first gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc BL21(DE3) 1 (3) Nuc BL21(DE3) 2 (4) Nuc BL21(DE3) 3 (5) Nuc BL21(DE3) 4 (7) Positive control (8) Negative control (9) DNA Ladder.

The wrong PCR program was used.

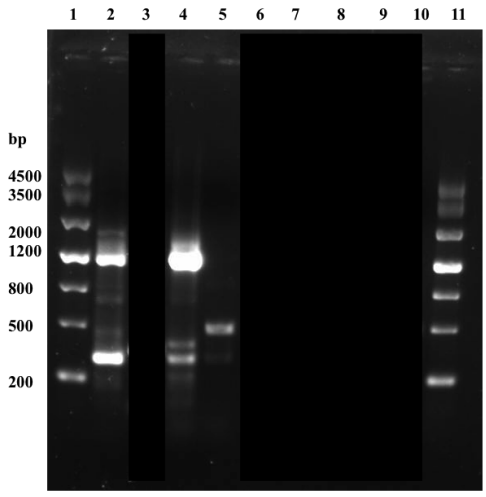

Figure 2: Result of the second gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) Nuc BL21(DE3) 1 (4) Positive control (5) Negative control.

Expected band at between 800-900 bp not present. The desired gene sequence has not been amplified.

Discussion and Troubleshooting

Step 7 in the Plasmid Extraction Kit Protocol by Qiagen was not performed as this is a recommended step. The washing step is recommended for endA+ strains which the *E.coli* strain BL21(DE3) is not. Eluting with dH_2O was recommended by Teresa Frisan, the secondary PI of the iGEM Stockholm 2016 team.

The desired PCR product have a size of approximately 800-900 bp. A corresponding band in the first gel can be seen in sample 1-3 (well 2-4) and very faint in sample 4 (well 5). But clearly, there are contaminations, especially at a size of 1200 bp. Since the wrong PCR program was used, an obvious source of error, no further troubleshooting was done. Instead, the PCR amplification was redone with the appropriate PCR program.

Since the result of the four samples from the first gel electrophoresis were similar, especially the first three, it was assumed that those contained the same DNA. Therefore, only Nuc BL21(DE3) 1 was re-amplified, representing all four samples. The result from the second gel electrophoresis shows that the expected band at between 800-900 bp is not present. Instead, contaminations similar to some of the ones found after the first PCR amplification can be seen. The pSB1K3-T7-Nuc plasmid is therefore probably not present in the sample and neither in the cells of the over night liquid culture.

2 3A Assembly (ligation) of pSB1A3-T7-Nuc with PCR analysis

Responsible

Ellinor Lindholm and Oscar He

Protocols used

3A Assembly
PCR Amplification (Q5)
Gel electrophoresis

Modifications and comments to protocols

3A Assembly:	Amount of vector per reaction	50 ng ($3.68 \cdot 10^{-14}$ mol)
	Volume of T4 DNA ligase	0.5 μl
	Volume of 10x T4 DNA ligase buffer	2 μl
	Total reaction volume	30 μl
	Ratio vector:insert	1:3
PCR Amplification (Q5):	1 μl DNA sample used in PCR mix.	
PCR Amplification (Q5) primers:	VR (reverse)	
	VF2 (forward)	

Experimental Setup

Table 4: Concentration and size of the parts used for ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1A3	2.3	2155
T7	6.6	32
Nuc 1.2 HQ	2.7	561

Table 5: Volumes of each component used in the ligation mix.

Assemble part	Volume [μl]
pSB1A3	21.74
T7	0.57
Nuc 1.2 HQ	14.17
T4 DNA Ligase	0.5
10x T4 DNA Ligase Buffer	3
ddH_2O	0

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{2}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{3}$$

Results and Conclusions

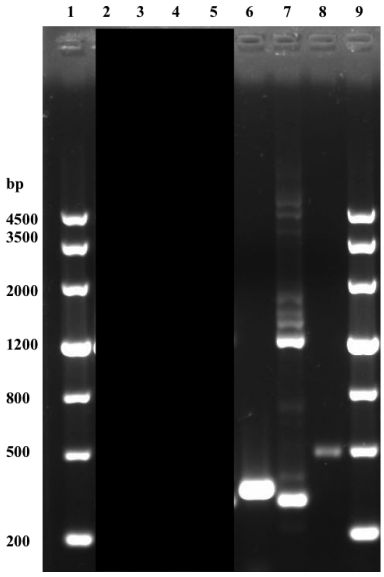

Figure 3: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (6) Nuc AmpR (7) Positive control (8) Negative control (9) DNA Ladder.

The wrong PCR program was used (ran at the same time as the first PCR amplification of extracted pSB1K3-T7-Nuc in the previous experiment).

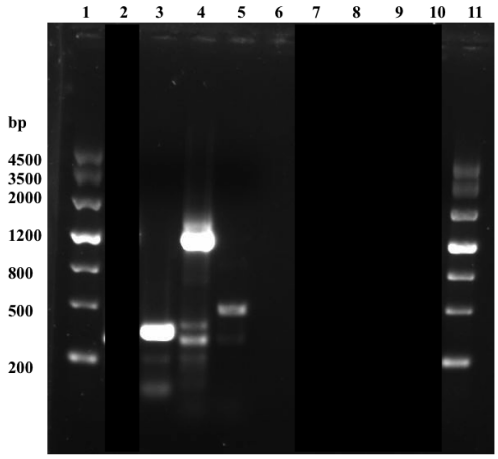

Figure 4: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (3) Nuc AmpR (4) Positive control (5) Negative control (11) DNA Ladder.

Expected band at between 800-900 bp not present. The desired gene sequence has not been amplified.

Discussion and Troubleshooting

The desired PCR product have a size of approximately 800-900 bp. Only one band can be seen at 300 bp in the first gel. Since the wrong PCR program was used, an obvious source of error, no further troubleshooting was done. Instead, the PCR amplification was redone with the appropriate PCR program.

The second gel also shows a strong band at 300 bp and therefore the desired plasmid is probably not present in the ligation product. The ligation was unsuccessful.

3 3A Assembly (ligation) of pSB1A3-T7-Nuc - second attempt

Responsible

Ellinor Lindholm and Oscar He

Protocols used

3A Assembly

Modifications and comments to protocols

3A Assembly:	Amount of vector per reaction	50 ng ($3.68 \cdot 10^{-14}$ mol)
	Volume of T4 DNA ligase	0.5 μl
	Volume of 10x T4 DNA ligase buffer	3 μl
	Total reaction volume (1)	30 μl
	Total reaction volume (2)	20 μl
	Ratio vector:insert	1:3

Two different nuclease and pSB1A3 samples were used for this ligation.

Experimental Setup

Table 6: Concentration and size of the parts used for the second attempt of ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1A3 (1)	2.3	2155
T7	6.6	32
Nuc 1.3 HQ SDS	2.7	561
pSB1A3 (2)	1.9	2155
T7	6.6	32
Nuc 2	5.2	561

Table 7: Volumes of each component used in the ligation mix.

Assemble part	Volume [μl]
pSB1A3 (1)	21.74
T7	0.517
Nuc 1.3 HQ SDS	14.17
T4 DNA Ligase	0.5
10x T4 DNA Ligase Buffer	3
ddH_2O	0
pSB1A3 (2)	26.37
T7	0.517
Nuc 2	7.36
T4 DNA Ligase	0.5
10x T4 DNA Ligase Buffer	2
ddH_2O	0

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{4}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{5}$$

Results and Conclusions

As no analytical experiment was conducted, no conclusion could be made regarding the ligation product itself. The success of the 3A assembly can be confirmed after transformation, see Section 4.

Discussion and Troubleshooting

Assuming the transformation of this ligation product is done correctly, if no colonies can be seen then the 3A assembly has failed.

4 Transformation of Top10 and BL21(DE3) cells with assembled pSB1A3-T7-Nuc

Responsible

Ellinor Lindholm and Oscar He

Protocols used

Transformation

Results and Conclusions

The transformation was unsuccessful as no colonies could be seen.

Discussion and Troubleshooting

The transformation most likely did not work because of unsuccessful 3A assembly. However, the transformation itself might not have worked.